requirements of 37 C.F.R. §1.824. Applicants respectfully request the entry of Exhibit D into file of the present application. In addition, applicants submit, herewith as **Exhibit E**, an initial computer readable form (CFR) copy of the "Sequence Listing" as required by 37 C.F.R. §1.825(d). Further, applicants submit, herewith as **Exhibit F**, a statement in accordance with 37 C.F.R. §1.821(f), certifying that the computer readable form containing the nucleic acid and/or amino acid sequences as required by 37 C.F.R. §1.821(e) contains the same information which was submitted as the "Sequence Listing" and contains no new matter.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone at the number provided below.

No fee, other than the enclosed $720.00 fee for a four-month extension of time is deemed necessary in connection with the filing of this Amendment. However, if any addition fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Mark A. Farley 10/16/02
John P. White Date
Reg. No. 28,678
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Reg. No. 33,170

Exhibit A

--30. (Amended) A vaccine which comprises a therapeutically effective amount of the protein encoded by [the] a nucleic acid [of claim 1] which comprises a nucleotide segment having a sequence encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.--

--33. (Amended) A vaccine which comprises a prophylactically effective amount of the protein encoded by [the] a nucleic acid [of claim 1] which comprises a nucleotide segment having a sequence encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.--

--46. (Amended) A mutant HIV-1 envelope protein which is encoded by [the] a nucleic acid [of any one of claims 3-18] which comprises a nucleotide segment having a sequence encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.--

10/24 AMD

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Exhibit B

30, 33, 44-46, 81, 82

In the claims:

--30. (2xAmended) A vaccine which comprises a therapeutically effective amount of the protein encoded by a nucleic acid which comprises a nucleotide segment having a sequence (SEQ ID NO:12-17) encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.--

--33. (2xAmended) A vaccine which comprises a prophylactically effective amount of the protein encoded by a nucleic acid which comprises a nucleotide segment having a sequence (SEQ ID NO:12-17) encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.--

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--46. (2xAmended) A mutant HIV-1 envelope protein which is encoded by a nucleic acid which comprises a nucleotide segment having a sequence (SEQ ID NO:12-17) encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.--

Exhibit C

In the specification:

Please replace the paragraph on page 22 lines 21-27 with the following paragraph:

--Figure 13

Nucleotide (A) (SEQ ID NO:12) and amino acid (B) (SEQ ID NO:13) sequences for HIV-1_{JR-FL} SOS gp140. The amino acid numbering system corresponds to that for wild-type JR-FL (Genbank Accession #U63632). The cysteine mutations are indicated in underlined bold type face.--

Please replace the paragraph that begins on page 22 line 37 and ends on page 23 line 5 with the following paragraph:

--Figure 14

Nucleotide (A) (SEQ ID NO:14) and amino acid (B) (SEQ ID NO:15) sequences for HIV-1_{JR-FL} ΔV1V2* SOS gp140. The amino acid numbering system corresponds to that for wild-type JR-FL (Genbank Accession #U63632). The cysteine mutations are indicated in underlined bold type face.--

Please replace the paragraph on page 23 lines 7-12 with the following paragraph:

--Figure 15

Nucleotide (A) (SEQ ID NO:16) and amino acid (B) (SEQ ID NO:17) sequences for HIV-1_{JR-FL} ΔV3 SOS gp140. The amino acid numbering system corresponds to that for wild-type JR-FL (Genbank Accession #U63632). The cysteine mutations are indicated in underlined bold type face.--

Please replace the paragraph on page 29 lines 20-28 with the following paragraph:

--As used herein, "C5 region" means the fifth conserved

sequence of amino acids in the gp120 glycoprotein. The C5 region includes the carboxy-terminal amino acids. In $HIV\text{-}1_{JR\text{-}FL}$ gp120, the unmodified C5 region consists of the amino acids GGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVQRE (SEQ ID NO:1). Amino acid residues 462-500 of the sequence set forth in figure 3A have this sequence. In other HIV isolates, the C5 region will comprise a homologous carboxy-terminal sequence of amino acids of similar length.--

Please replace the paragraph that begins on page 30 line 23 and ends on page 31 line 7 with the following paragraph:

--As used herein, "C1 region" means the first conserved sequence of amino acids in the mature gp120 glycoprotein. The C1 region includes the amino-terminal amino acids. In $HIV_{JR\text{-}FL}$, the C1 region consists of the amino acids VEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPN PQEVVLENVTEHFNMWKNNMVEQMQEDIISLWDQSLKPCVKLTPLCVTLN (SEQ ID NO:2). Amino acid resides 30-130 of the sequence set forth in in figure 3A have this sequence. In other HIV isolates, the C1 region will comprise a homologous amino-terminal sequence of amino acids of similar length. W44C and P600C mutations are as defined above for A492 and T596 mutations. Because of the sequence variability of HIV, W44 and P600 will not be at positions 44 and 600 in all HIV isolates. In other HIV isolates, homologous, non-cysteine amino acids may also be present in the place of the tryptophan and proline. This invention encompasses cysteine mutations in such amino acids, which can be readily identified in other HIV isolates by those skilled in the art.--

Please replace the paragraph that begins on page 55 line 27 and ends on page 56 line 21 with the following paragraph:

--*Wild-type gp140s (gp140WT)* The gp140 coding sequences were amplified using the polymerase chain reaction (PCR) from full-length molecular clones of the HIV-1 isolates JR-FL, DH123, Gun-1, 89.6, NL4-3 and HxB2. The 5' primer used

was designated Kpn1env (5'-GTCTATTATGGGGTACCTGTGTGGAA AGAAGC-3')(SEQ ID NO:3) while the 3' primer was BstB1env (5'-CGCAGACGCAGATTCGAATTAATACCACAGCCAGTT-3')(SEQ ID NO:4). PCR was performed under stringent conditions to limit the extent of *Taq* polymerase-introduced error. The PCR products were digested with the restriction enzymes *Kpn*1 and *Xho*1 and purified by agarose gel electrophoresis. Plasmid PPI4-tPA-gp120$_{JR-FL}$ was also digested with the two restriction enzymes and the large fragment (vector) was similarly gel-purified. The PPI4-tPA-gp120$_{JR-FL}$ expression vector has been described previously (Hasel and Maddon, U.S. Patents #5886163 and 5869624). Ligations of insert and vector were carried out overnight at room temperature. DH5αF'Q10 bacteria were transformed with 1/20 of each ligation. Colonies were screened directly by PCR to determine if they were transformed with vector containing the insert. DNA from three positive clones of each construct were purified using a plasmid preparation kit (Qiagen, Valencia, CA) and both strands of the entire gp160 were sequenced. By way of example, pPPI4-gp140WT$_{JR-FL}$ and pPPI4-gp140WT$_{DH123}$ refer to vectors expressing wild-type, cleavable gp140s derived from HIV-1$_{JR-FL}$ and HIV-1$_{DH123}$, respectively.--

Please replace the paragraph on page 56 lines 22-33 with the following paragraph:

--*gp140UNC* A gp120-gp41 cleavage site mutant of JR-FL gp140 was generated by substitutions within the REKR motif at the gp120 C-terminus, as described previously (Earl et al., Proc. Natl. Acad. Sci. USA 87:648, 1990). The deletions were made by site-directed mutagenesis using the mutagenic primers 5'140M (5'-CTACGACTTCGTCTCCGCCTT CGACTACGGGGAATAGGAGCTGTGTTCCTTGGGTTCTTG-3')(SEQ ID NO:5) and 3'gp140M (sequence conjunction with Kpn1env and BstB1env 5'-TCGAAGGCGGAGACGAAGTCGTAGCCGCAGTGCCTTGGTGG GTGCTACTCCTAATGGTTC-3')(SEQ ID NO:6). In conjunction with

Kpn1env and BstB1, the PCR product was digested with *Kpn*1 and *Bst*B1 and subcloned into pPPI4 as described above.--

Please replace the paragraph on page 57 lines 9-35 with the following paragraph:

--PCR amplification using DGKPN5'PPI4 and 5JV1V2-B (5'-GTCTATTATGGGGTACCTGTGTGGAAAGAAGC-3')(SEQ ID NO:7) on a ΔV1 template and subsequent digestion by *Kpn*1 and *Bam*H1 generated a 292bp fragment lacking the sequences encoding the V1 loop. This fragment was cloned into a plasmid lacking the sequences for the V2 loop using the Kpn1 and BamH1 restriction sites. The resulting plasmid was designated ΔV1V2' and contained a Gly-Ala-Gly sequences in place of both D132-K152 and F156-I191. Envs lacking the V1, V2 and V3 loops were generated in a similar way using a fragment generated by PCR on a ΔV3 template with primers 3JV2-B (5'-GTCTGAGTCGGATCCTGTGACACCTCAGTCATTACACAG-3')(SEQ ID NO:8) and H6NEW (5'CTCGAGTCTTCGAATTAGTGATGGGT GATGGTGATGATACCACAGCCATTTTGTTATGTC-3')(SEQ ID NO:9). The fragment was cloned into ΔV1V2', using BamH1 and BstB1. The resulting env construct was named ΔV1V2'V3. The glycoproteins encoded by the ΔV1V2' and ΔV1V2'V3 plasmids encode a short sequence of amino acids spanning C125 to C130. These sequences were removed using mutagenic primers that replace T127-I191 with a Gly-Ala-Gly sequence. We performed PCR amplification with primers 3'DV1V2STU1 (5'-G G C T C A A A G G A T A T C T T T G G A C A G G C C T G T GTAATGACTGAGGTGTCACATCCTGCACCACAGAGTGGGGTTAATTTTACACATGGC-3')(SEQ ID NO:10) and DGKPN5'PPI4, digested the resulting fragment by *Stu*1 and *Kpn*1 and cloned it in a PPI4 gp140 vector. The resulting gp140 was named ΔV1V2*. In an analogous manner ΔV1V2*V3 was constructed. The amino acid substitutions are shown schematically in Figure 10.--

Please replace the paragraph on page 59 lines 8-33 with the following paragraph:

--The concentration of gp120 and gp140 proteins in 293T cell supernatants was measured by ELISA (Binley et al. J. Virol 71:2799, 1997). Briefly, Immulon II ELISA plates

(Dynatech Laboratories, Inc.) were coated for 16-20 hr at 4 °C with a polyclonal sheep antibody that recognizes the carboxy-terminal sequence of gp120 (APTKAKRRVVQREKR) (SEQ ID NO:11). The plate was washed with tris buffered saline (TBS) and then blocked with 2% nonfat milk in TBS. Cell supernatants (100 µL) were added in a range of dilutions in tris buffered saline containing 10% fetal bovine serum. The plate was incubated for 1 hr at ambient temperature and washed with TBS. Anti-gp120 or anti-gp41 antibody was then added for an additional hour. The plate was washed with TBS, and the amount of bound antibody is detected using alkaline phosphatase conjugated goat anti-human IgG or goat anti-mouse IgG. Alternatively, biotinylated reporter Abs are used according to the same procedure and detected using a streptavidin-AP conjugate. In either case, AP activity is measured using the AMPAK kit (DAKO) according to the manufacturer's instructions. To examine the reactivity of denatured HIV envelope proteins, the cell supernatants were boiled for 5 minutes in the presence of 1% of the detergents sodium dodecyl sulfate and NP-40 prior to loading onto ELISA plates in a range of dilutions. Purified recombinant JR-FL gp120 was used as a reference standard.--